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Concentration-Dependent Mobility of Chlorfenvinphos in Isolated Plant Cuticles

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Abstract: The mobility of chlorfenvinphos in isolated pear (*Pyrus communis* cv. Bartlett) leaf cuticular membranes (CM) was studied as a function of concentration of chlorfenvinphos sorbed in the cuticle. Mobilities of chlorfenvinphos increased approximately 9-fold when the amount sorbed increased from 1 to 100 $\mu g\ cm^{-2}$ pear leaf cuticle. From the amounts per area, average volume fractions of chlorfenvinphos in the cuticle were calculated ranging from 2×10^{-3} to $5{\cdot}1 \times 10^{-2}$. The increase in mobilities was steepest at the lower and levelled off at higher volume fractions. This correlation could be described for the whole range of volume fractions investigated by an equation which assumes homogeneously dispersed chlorfenvinphos. Temperature dependence of mobilities was studied at 17, 25 and 35°C and chlorfenvinphos volume fractions of $5{\cdot}5 \times 10^{-3}$ and 0·12, respectively. Arrhenius graphs were linear for both volume fractions, showing that cuticles did not undergo a phase transition due to the high amount of sorbed chlorfenvinphos. However, at a volume fraction of 0·12, the activation energy of diffusion, E_D, was significantly lower (83·6 kJ mol^{-1}) than at $5{\cdot}5 \times 10^{-3}$ (135 kJ mol^{-1}). We interpret these findings as evidence for a plasticising effect on cuticular waxes by chlorfenvinphos. So far, such an effect had been demonstrated only for certain adjuvants (ethoxylated alcohols) but not for active ingredients. Chlorfenvinphos not only increased its own mobility in pear leaf cuticles, but also that of 2,4-D in *Citrus* leaf cuticles. This would be expected if plasticising of waxes was the sole mechanism responsible for increased mobilities. From these data we predict that permeabilities of cuticles to chlorfenvinphos are not constant. Depending on temperature as well as types and amounts of adjuvants, rates of foliar penetration of chlorfenvinphos can be higher if its concentration in the spray liquid is increased.

Key words: diffusion, foliar uptake, permeability, plasticising, swelling

1 INTRODUCTION

Systemic pesticides applied to foliage must penetrate the cuticle before they can become biologically effective.[1] Rapid uptake is desirable and various adjuvants included in formulations are known to increase efficacy.[2] This could be due in part to faster penetration.[3–8] Rates of foliar uptake depend on the barrier properties of the plant cuticle which covers all primary organs of higher plants.[9] Studies using isolated plant cuticles can help to understand better the mechanistic aspects of foliar penetration and how external factors such as temperature and adjuvants influence diffusion across cuticles.[10–14] Two adjuvant effects on rates of foliar uptake must be distinguished: (i) alterations in the driving forces of uptake due to changes in wetting, retention, partitioning and the physical state of the active ingredient on the plant surface (ii) acceleration in foliar uptake by increasing the mobility of the active ingredient in the plant cuticle. These effects depend on the specific properties of the adjuvant and its concentration in the cuticle.[14] Many adjuvants are surfactants, but surface activity or ethoxylation are not essential properties of accelerators, since concentration-dependent diffusion coefficients in polymers have been observed with many organic vapours[15,16] and plasticisers.[17,18]

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Solute mobilities in plant cuticles can be measured by unilateral desorption from the outer surface (UDOS). Using this method it is also possible to measure effects of adjuvants on the mobility of active ingredients.[14] In a recent study concerning interactions between active ingredients during cuticular penetration, it was found that high concentrations of permethrin and chlorfenvinphos increased the mobility of 2,4-D in *Citrus* leaf cuticular membranes (CM).[19] This observation suggested that mobilities of these compounds should depend on concentration. Results obtained with chlorfenvinphos are reported here.

2 MATERIALS AND METHODS

2.1 Cuticular membranes

Adaxial astomatous cuticular membranes were isolated enzymatically from mature leaves of bitter orange (*Citrus aurantium* L.) and pear (*Pyrus communis* L. cv. Bartlett) as described elsewhere.[20] Bitter orange plants were grown in growth chambers, while fully expanded pear leaves were taken from trees in an orchard in Bavaria in July 1992. Isolated cuticles were air-dried and stored for at least four weeks at about 8°C prior to use.

2.2 Chemicals

2,4-Dichlorophenoxy-[1-^{14}C]acetic acid (2,4-D; specific activity 329 MBq mmol^{-1}, Amersham Buchler, Braunschweig, Germany) and 2-chloro-1-([U-^{14}C]-2,4-dichlorophenyl)vinyl diethyl phosphate (chlorfenvinphos, 303 MBq mmol^{-1}, Shell Research Ltd, Sittingbourne, UK) were used in this study. Radiochemical purities of the compounds were better than 98%. Both pesticides are lipophilic and have cuticle/water partition coefficients (K_{CW}) of 770 (non-ionised form of 2,4-D) and 1580 (chlorfenvinphos).[19] Chlorfenvinphos was dissolved in water containing 25 to 40% ethanol (w/w), depending on concentration. 2,4-D is a weak acid, pK_a 2·73,[21] and it was dissolved in 0·01 M lactic acid buffer adjusted to pH 3 with potassium hydroxide. After loading with radio-labelled solutes, the 2,4-D concentration in the *Citrus* CM was 4·5 mg g^{-1} and the lowest chlorfenvinphos concentration used in pear leaf CM was 3·85 mg g^{-1}.

The lowest concentration of chlorfenvinphos in the donor solution was 0·02 g litre^{-1}. Higher concentrations were obtained by mixing [^{14}C] chlorfenvinphos with non-radioactive compound obtained from Shell Research Ltd, Sittingbourne, UK (99·3% purity). Droplets (50 µl) of the donor solutions were applied to the morphological inner side of the cuticles to give doses of 1, 5, 10, 50 and 100 µg cm^{-2}.

2.3 Experimental

Mobilities of compounds were determined using unilateral desorption from the outer surface (UDOS). Details of the method have been described elsewhere[22] and only the principles will be described here. Briefly, cuticles were inserted between lid and desorption chamber with the morphological outer surface facing the chamber interior. The donor solutions containing the radio-labelled pesticide were applied as 50 µl droplets to the centre of the morphological inner surface of the CM (one droplet per cuticle). During evaporation of ethanol and water (3–6 h) the lipophilic compounds were quantitatively sorbed in the cuticle. Desorption from the outer surface was started the next day by pipetting an aqueous soybean lecithin suspension (10 g litre^{-1}; PLS) through a sampling port into the chamber. For calculating rate constants (k^*) from UDOS data it is assumed that the concentration of the desorbed compound in the receiver is zero. This assumption is fulfilled if PLS is used as desorption medium for lipophilic neutral solutes, which partition into the liposomes.[23] During desorption the chambers were shaken horizontally while standing with the lids facing downward in wells of a thermostated (25°C) aluminium block. At predetermined time intervals the desorption medium (PLS) was withdrawn and replaced by a fresh one. After the last desorption step, the part of the CM exposed to the receiver solution was cut out and the residual radioactivity in it was extracted using scintillation cocktail (Aquasafe 500, Zinsser, Frankfurt, Germany). Radioactivity in the desorption media and CM were assayed using a liquid scintillation counter (Packard CA 2000 counter, Downers Grove, IL, USA).

The temperature effect on mobility was determined for each CM separately using the method of paired comparisons, by carrying out desorption in successive stages starting at 17°C and progressing to higher temperatures of 25 and 35°C. After changing the temperature of the aluminium block the desorption chambers (including CM and solutions) attained the desired temperature within 5 to 10 min. This was ascertained by directly measuring the temperature of the solutions in the chambers. The temperatures remained constant to $\pm$ 0·8°C over the total area of the aluminium block. Sampling of donor solutions was started as soon as temperature was constant.

2.4 Rate constants

The ratio of the concentration, C_t, of the radio-labelled solute at the time t in the CM to the original concentration C_0 at $t = 0$ decreased according to first-order desorption kinetics:

$$C_t/C_0 = \exp(-k^*t) \quad (1)$$

with k^* being the first-order rate constant of desorption. The asterisk denotes that this rate constant is independent of the partition coefficient K.[22] Plotting $-\ln(C_t/C_0)$ against t yielded straight lines with slope k^*. Since the volume of the CM in which solutes are sorbed is constant, the ratio C_t/C_0 is equivalent to the expression $1 - M_t/M_0$, where M_t is the amount of substance desorbed at time t. M_0 is the amount sorbed initially in the CM and it was calculated by summation of the amounts desorbed plus the amount remaining in the CM after termination of desorption. Between 10 and 20 CM (replications) were used for each species/compound combination at constant temperature. The effect of temperature on mobility of chlorfenvinphos was studied using larger populations of 30 CM and the same CM were used for all temperatures (paired observations) starting at the lowest temperature. Arithmetic means and confidence intervals were calculated from the logarithmic values of rate constants k^*.

3 RESULTS

3.1 Effects of chlorfenvinphos in the desorption medium on solute mobility

Radio-labelled 2,4-D sorbed in *Citrus* CM was initially desorbed using PLS. PLS is an inert desorption medium, as it does not penetrate the CM and has no effect on solute mobility in cuticles.[22,24,25] The slope up to the arrow (Fig. 1A) therefore characterises solute mobility in the native, unaltered waxes of the cuticle. In order to test if chlorfenvinphos (CFP) changes 2,4-D mobility in the cuticles, desorption was continued using PLS containing non-radioactive chlorfenvinphos at the time marked by an arrow. The slopes, that is the rate constants k^*, increased immediately after changing the desorption medium and after one day (first sample) already 87–92% of sorbed 2,4-D had penetrated (Fig. 1A). The ratio of the rate constants of desorption measured in the presence and absence of an adjuvant is a quantitative measure of the effect of this adjuvant on solute mobility.[25] This ratio is called effect. Maximum effect means that those rate constants were used in calculations where slopes are steepest. In the present case, maximum rates were observed during the time interval 48 to 75 h (Fig. 1A). Since each CM is both control (desorption with inert PLS yielding k^*_{pls}) and treatment (desorption with PLS + chlorfenvinphos), effects can be calculated for each CM separately. Maximum effects were plotted against the reciprocal of the rate constants measured using PLS (k^*_{pls}) and a linear relationship was obtained (Fig. 1B). This response graph shows that the effect of chlorfenvinphos was greatest with CM having a low intrinsic solute mobility (k^*_{pls}). The mean increase in 2,4-D mobility due to sorbed chlorfenvinphos was 22-fold.

A similar experiment was conducted using radio-labelled chlorfenvinphos (1·5 $\mu g\ cm^{-2}$) as solute and pear leaf CM. On changing desorption medium from PLS to PLS + chlorfenvinphos (arrow), slopes also increased greatly, but with CM having low intrinsic mobilities it took about 24 h before maximum rates of desorption were established (Fig. 2A). The response graph was again linear (Fig. 2B) but both values of $1/k^*_{pls}$ and maximum effects were larger than observed with 2,4-D and *Citrus* CM. The mean increase in chlorfenvinphos mobility in pear leaf CM was 35-fold.

3.2 Concentration-dependent penetration of sorbed chlorfenvinphos

To study concentration dependence of rate constants, pear leaf CM were loaded with mixtures of radio-labelled and non-labelled chlorfenvinphos in amounts

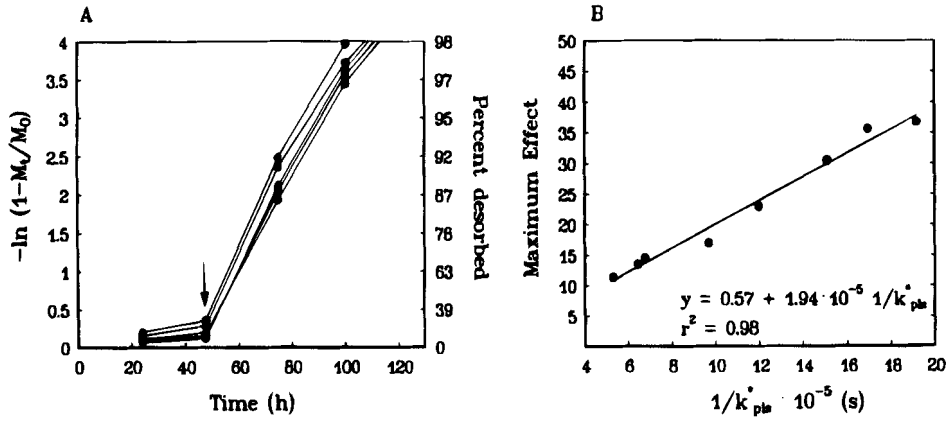

Fig. 1. (A) Time course of desorption of 2,4-D from *Citrus* CM with PLS only (0 to 48 h) or with chlorfenvinphos (0·025 M) dissolved in 1% PLS (after 48 h). The arrow indicates time of chlorfenvinphos addition. The lines represent desorption from five individual CM. (B) Dependence of the maximum effect on initial rate constants measured using PLS as desorption medium (k^*_{pls}). The maximum effect is the ratio of maximum rate constants of desorption (interval 48 to 75 h) over k^*_{pls}. This ratio was calculated for each CM separately.

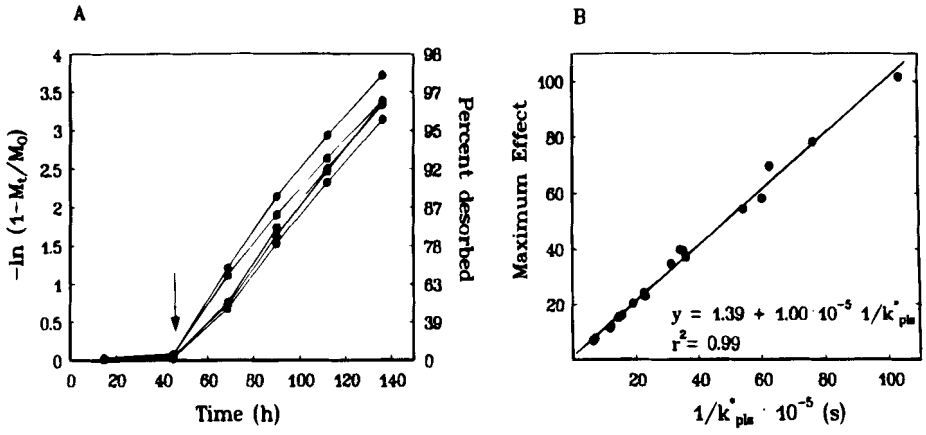

Fig. 2. (A) Time course of desorption of radio-labelled chlorfenvinphos from pear leaf CM with PLS only (0 to 44 h) or with chlorfenvinphos (0·025 M) dissolved in 1% PLS (after 44 h). The arrow indicates time of chlorfenvinphos addition. The lines represent desorption from five individual CM. (B) Dependence of the maximum effect on initial rate constants measured using PLS as desorption medium (k^*_{pls}). The maximum effect is the ratio of maximum rate constants of desorption (interval 69 to 90 h) over k^*_{pls}. This ratio was calculated for each CM separately.

ranging from 1 to 100 $\mu g\ cm^{-2}$. The amounts of chlorfenvinphos sorbed in the cuticle varied by two orders of magnitude and reached very high levels (1 cm^2 of pear leaf cuticle has a mass of approximately 260 μg) and, under these conditions, penetrant concentration is best described in terms of volume fractions.[16] The volume fraction is the ratio of the penetrant volume to the sum of penetrant and polymer volumes. Volume fractions were calculated using McGowan's characteristic volume[26] of chlorfenvinphos ($V_x = 233\ cm^3\ mol^{-1}$) and the mass and density ($1{\cdot}1 \times 10^3\ kg\ m^{-3}$) of the pear cuticle underlying the droplet.[27] Initial volume fractions of chlorfenvinphos ranged from $2{\cdot}74 \times 10^{-3}$ to 0·215. In this experiment the cuticles already contained sorbed chlorfenvinphos at the beginning of the experiment and desorption was carried out using 1% PLS with no CFP added.

At low volume fractions, desorption graphs were linear after about 24 h, while, with the two highest volume fractions, linearity was obtained after about 48 h (Fig. 3A). Slopes (i.e. rate constants k^*) increased with increasing volume fractions of chlorfenvinphos initially sorbed in the cuticles. The mean rate constants between minimum and maximum volume fractions differed by a factor of 8·5 (Table 1). The lines of the desorption graphs passed through the origin only with the lowest volume fraction. Positive y-intercepts were obtained with higher volume fractions, indicating a two-phase process. Because chlorfenvinphos was eliminated from the cuticles during desorption, initial volume fractions only describe the situation at $t = 0$. With increasing time, volume fractions decrease. Since we wished to study the effect of sorbed chlorfenvinphos on the mobility of the compound in cuticles, the corresponding volume fractions of chlorfenvinphos were required at the time when desorption graphs were linear. These were calculated using the actual amounts in the cuticles 65 h after starting desorption and are given on the right side in Fig. 3A. When rate constants (log k^*) for the linear portions of the desorption graphs were plotted against these volume fractions a saturation type curve was obtained (Fig. 3B).

3.3 Activation energies of diffusion as affected by chlorfenvinphos concentration

The temperature dependence of chlorfenvinphos mobility in cuticles was studied using a temperature range of 17–35°C, because cuticles do not appear to undergo phase transition at these temperatures.[27,28] Chlorfenvinphos was applied at two different volume fractions. The initial volume fractions were $5{\cdot}5 \times 10^{-3}$ and 0·12. The lower is the minimum amount of radioactivity required for measurements, while the higher is identical

TABLE 1

Rate Constants of Desorption, k^*, Ratios of Maximum to Minimum Rates and Activation Energies of Diffusion E_D for Different Volume Fractions of Chlorfenvinphos Sorbed in Pear Leaf CM

Average volume fraction[a]	k^* $(s^{-1})^b$ $\times 10^{-6}$	95% CI^b $\times 10^{-6}$	$\frac{k^*(max.)}{k^*(min.)}$	E_D $(kJ\ mol^{-1})$
0·002	0·60	0·25	18	135·0[c]
0·009	1·55	0·66	22	
0·014	2·19	0·62	5·9	
0·034	4·27	0·71	3·0	
0·051	5·13	0·72	2·2	83·6[d]

[a] Calculated for a mass per area of the cuticle of 260 $\mu g\ cm^{-2}$ and after 65 h desorption.

[b] Arithmetic mean and 95% average confidence interval were calculated from the logarithm of k^*.

[c] For a chlorfenvinphos volume fraction of $5{\cdot}5 \times 10^{-3}$ (from Ref. 40).

[d] For an initial chlorfenvinphos volume fraction of 0·12.

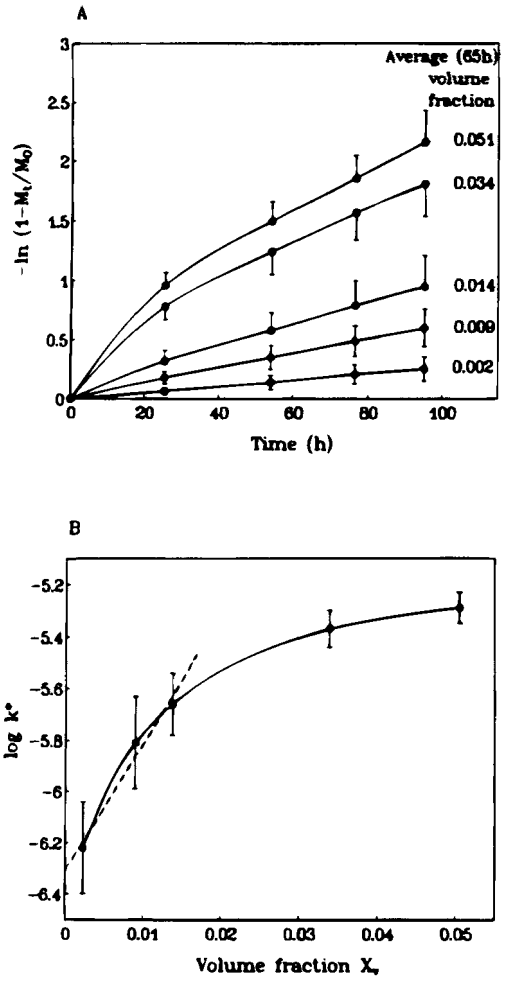

Fig. 3. (A) Time course of desorption of chlorfenvinphos from pear leaf CM at different volume fractions of chlorfenvinphos sorbed in the cuticle. Each point represents the arithmetic mean from 16 to 19 CM and the 95% confidence interval. (B) Dependence of rate constants of desorption of chlorfenvinphos from pear leaf CM on volume fraction of chlorfenvinphos sorbed in the CM. Each point represents the arithmetic mean of log k^* from 16 to 19 CM and the 95% confidence interval.

with the second highest initial volume fraction of the concentration series above (see section 3.2). The desorption graphs for five selected cuticles including the most and least permeable ones at 17, 25 and 35°C are shown in Fig. 4. The initial volume fraction of chlorfenvinphos amounted to 0·12. Desorption was constant immediately after establishing a new temperature, even though much shorter time intervals were used at 25 and 35°C. The time course of desorption at the lower volume fraction was very similar and is not shown. The concentration of chlorfenvinphos in the cuticle decreased during the experiment, i.e. the volume fractions of chlorfenvinphos were lower at 25 and 35°C than at 17°C. With most CM only 25% or less of the initial amount of chlorfenvinphos was desorbed during the course of the experiment, but with some CM having high solute mobilities up to 48% was desorbed (Fig. 4, ordinate on the right).

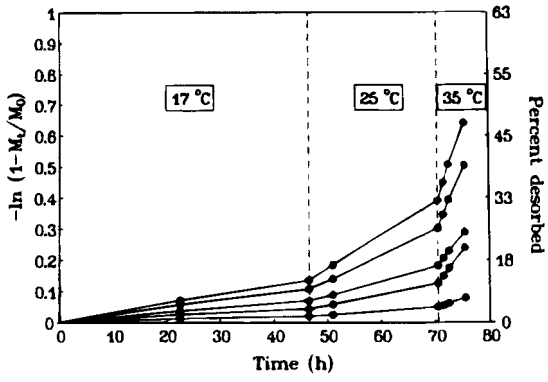

Fig. 4. Time course of desorption of chlorfenvinphos with PLS from pear leaf CM at different temperatures. Results for five individual CM including the most and least permeable ones are shown.

Arrhenius graphs were linear for both volume fractions of chlorfenvinphos, but slopes were steeper with the lower volume fraction (Fig. 5). The activation energy of diffusion (E_D) can be calculated from the slopes and the gas constant R using eqn (2):

$$E_D = (-1) \,.\, \text{slope} \,.\, R \,.\, 2{\cdot}3 \qquad (2)$$

Activation energies of diffusion at volume fractions of $5{\cdot}5 \times 10^{-3}$ and 0·12 were 135 and 83·6 kJ mol^{-1}, respectively. The difference of about 50 kJ mol^{-1} is significant at the 1% level. The energy of activation obtained for the lowest volume fraction of chlorfenvinphos describes temperature effects on diffusion of a molecule in the native, unaltered cuticular membrane, since, at this low volume fraction, chlorfenvinphos has no significant accelerating effect. In the experiment with the high volume fraction of chlorfenvinphos mobility is affected by both temperature and chlorfenvinphos. The effect of the latter on mobility was not entirely constant

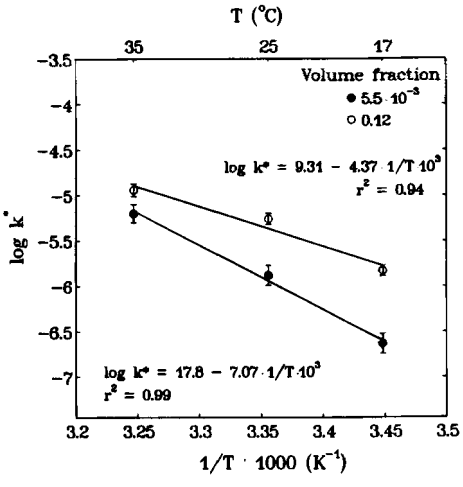

Fig. 5. Arrhenius plots of rate constants of desorption of chlorfenvinphos from pear leaf CM. Each point represents the arithmetic mean of log k^* from 30 cuticles. Bars represent 95% confidence intervals.

at all three temperatures, since its volume fractions decreased somewhat while going from low to high temperatures.

4 DISCUSSION

We have shown that chlorfenvinphos increases the mobility of 2,4-D in *Citrus* leaf CM (Fig. 1) and its own mobility independent of the methods of application (Figs 2 and 3). Compounds which increase solute mobilities in cuticles have been termed accelerators in order to distinguish this highly specific effect on the properties of cuticles and waxes from effects of adjuvants on biological activity and performance of plant protection agents, where the exact mechanism of action is usually not known.[14,22,29]

With surfactants it has been shown that this accelerating effect requires the presence of surfactants in the cuticle and in cuticular waxes.[22,29,30] The magnitude of the effect increases with increasing concentration in the cuticle and the effects therefore depend on the solubility of the accelerator in the cuticle (cuticle/water partition coefficient) and on the concentration of accelerator in the medium surrounding the cuticle. We have shown here for the first time that accelerating activity in cuticles is not limited to adjuvants or surfactants. Active ingredients themselves can also have accelerating properties. Figure 3B indicates that rates of foliar uptake of chlorfenvinphos may depend on concentration in the spray liquid, such that higher rates will be observed with increasing concentrations. However, the magnitude of this effect will vary depending on the concentration of the compound (Fig. 3), on temperature (Fig. 5) and on the presence of other accelerator adjuvants as components to the formulation. If chlorfenvinphos is sprayed together with other active ingredients, their rates of penetration might also be increased. Since chlorfenvinphos is a liquid at ambient temperatures, rates of penetration will not be reduced by the formation of solid spray residues on the leaf surface.

Chlorfenvinphos is an oily organic liquid (melting range -19 to -23°C) and it can serve both as solvent and as plasticiser. It is well-known that certain oils increase the foliar uptake of active ingredients if used as adjuvants.[31] It has also been shown that methyl esters of seed oils increase the rate of uptake of diclofop-methyl in maize leaves by approximately 10-fold.[32] The uptake of haloxyfop-methyl into yellow foxtail leaves was similarly dramatically increased by the addition of a crop oil concentrate.[33] In that study haloxyfop-methyl was applied to the leaves as 5-μl droplets with concentrate amounts ranging from 0·5 to 150 μg per droplet. A plot of percentage uptake of haloxyfop-methyl against amount of crop oil concentrate applied showed that improvement of uptake was relatively higher at low amounts and levelled off above 15 μg. This is very similar to the result in the present study (Fig. 3B). In the studies cited above, rates of uptake were measured rather than rates of diffusion. Since rates of uptake depend on both permeabilities of cuticles and on driving forces, adjuvant effects observed in these studies[31–33] may have been due in part to their effects on driving forces.[14]

4.1 Effect of sorbed chlorfenvinphos on solute mobility

Comparing Figs 1 and 3, it is evident that the accelerating properties of CFP depend on plant species, compound and on the method of application. The average effect was smaller with 2,4-D in *Citrus* than with chlorfenvinphos in pear leaf CM. This may seem strange at first sight, since the permeability of pear leaf CM is usually somewhat higher than that of *Citrus* CM, both for water[22] and organic solutes having the same size.[19] However, we have recently shown that solute mobility in plant cuticles and in cuticular waxes decreases rapidly with increasing size such that the mobility of solutes depends exponentially on their size.[14,19] Molar volumes of 2,4-D (138 $cm^3\ mol^{-1}$) and chlorfenvinphos (233 $cm^3\ mol^{-1}$) differ considerably and this accounts for the fact that k^*_{pls} was larger for 2,4-D in *Citrus* CM than for chlorfenvinphos in pear leaf CM. Interestingly, differences in size selectivity of *Citrus* and pear CM were only minor, while the mobility of the same compound was considerably lower in *Citrus* CM[19] and differences in path lengths across cuticles (tortuosity) probably account for this fact.

Acceleration by chlorfenvinphos was larger for that compound (Fig. 2) than for 2,4-D (Fig. 1). There is a simple explanation for this, because the effect of a given accelerator increases with increasing $1/k^*_{pls}$, and it does not matter if $1/k^*_{pls}$ is large because the CM is a good barrier, or because the solute has a large molar volume.[34] Desorption was carried out for both solutes using a mixture of 1% PLS + chlorfenvinphos, and equilibrium concentrations in cuticles will have been very similar, since cuticle/water partition coefficients for a given compound do not differ much among cuticles from different plant species.[9] Thus, differences in chlorfenvinphos concentrations in *Citrus* and pear leaf cuticles were probably very small in these experiments and are not likely to have contributed greatly to the differences in acceleration of diffusion of 2,4-D and chlorfenvinphos. The linearity of the plots of maximum effect versus $1/k^*_{pls}$ (Figs 1B and 2B) means that differences in solute mobility in different cuticles disappear if high amounts of chlorfenvinphos are sorbed. This is also indicated by a decreasing ratio of maximum and minimum rate constants for increasing volume fractions of chlorfenvinphos (Table 1). Rate constants for the desorption of radio-labelled chlorfenvinphos with

1%PLS + chlorfenvinphos from pear CM (Fig. 2) differ only by a factor of 1·1 and the arithmetic mean amounts to $1{\cdot}05 \times 10^{-5}\ s^{-1}$. This value is identical with the mean rate constant for the highest volume fraction of the concentration series in the first (0–24 h) sampling interval (Fig. 3A).

4.2 Activation energies of diffusion as influenced by solute concentration

In the remainder of this discussion we shall focus on mechanistic aspects of acceleration by chlorfenvinphos. We shall discuss two hypotheses and test them on the basis of available evidence. (i) Chlorfenvinphos sorbed in the cuticle in sufficient amounts may induce irreversible structural changes in the cuticular membrane. For instance, the compound may induce phase transitions or change the transition temperatures of the cutin polymer and/or the cuticular waxes. This is the mode of action of many plasticisers used in high polymers.[35,36] (ii) The increase in solute mobility may be solely caused by plasticising (swelling) of the cuticle or its limiting barrier. Increasing amounts of chlorfenvinphos could decrease the cohesive energies of chain segments in the polymer and/or of amorphous wax regions,[37] thereby lowering the viscosity.[17]

Arrhenius plots for the two different volume fractions of chlorfenvinphos were linear (Fig. 5), but the slope is smaller when the chlorfenvinphos volume fraction is high. Linearity of Arrhenius plots makes phase transitions in the temperature range studied unlikely and indicates that chlorfenvinphos did not produce structural modifications in the cuticle—e.g. a change in the ratio of crystalline to amorphous waxes. The significant difference in activation energies of diffusion of approx. 50 kJ mol^{-1} (Table 1) suggests that a plasticising or swelling effect was operative. The presence of chlorfenvinphos leads to a new phase with decreased cohesive energies of neighbouring chains of waxes and/or cutin causing increased diffusion coefficients of solutes with a reduced temperature dependence of the diffusion process.

If chlorfenvinphos acted only by swelling, leaving the original structure of the wax/cutin composite unchanged, one would expect the effect to be fully reversible once the compound was removed completely. The desorption graphs shown in Fig. 3A exhibit good linearity up to the end, even though a substantial fraction (more than 80% at the high volume fractions), of the chlorfenvinphos sorbed initially had been desorbed at the end of the experiment. This could be interpreted as evidence for irreversible effects of CFP on wax structure (memory or history effect). However, plant cuticles are highly asymmetrical membranes and the waxy barrier limiting rates of diffusion is confined to a thin layer at the morphological outer surface of the CM.[14] The largest part of the CM underneath this limiting skin serves as a sorption compartment and most of the chlorfenvinphos applied initially ($t = 0$), will be confined to this large sorption compartment.[9] Limiting skin and sorption compartment differ greatly not only in mass but also in partition coefficients, since wax/water partition coefficients have been shown to be smaller by factors of 10 to 20 than cuticle/water partition coefficients.[38] From this scenario it can be deduced that both amounts and concentrations of chlorfenvinphos in the wax of the limiting barrier will be much smaller than the average concentrations. During desorption with PLS, the chlorfenvinphos molecules will be pulled through the limiting barrier. These molecules that diffuse from the limiting barrier into the PLS will be replaced by others from the sorption compartment. In this way a relatively constant steady-state chlorfenvinphos concentration in the waxes of the limiting barrier could be maintained for a long time, even though the average concentration apparently decreased. This argument is plausible but we admit that the question about reversibility of chlorfenvinphos effects cannot be answered at this time. Due to heterogeneity of cuticles we have no information about local chlorfenvinphos concentrations; only average concentrations are known. Furthermore, we cannot exclude history dependence of the observed effects. History dependence of diffusion coefficients is a common phenomenon and it is difficult to measure even with pure polymers.[15,16,39]

Although we do not know the absolute amounts of chlorfenvinphos in the limiting skin, at constant temperature they are proportional to the whole amounts applied. Thus, it is reasonable to analyse the concentration dependence by relating the observed mobilities to the average volume fractions of chlorfenvinphos in the cuticle as can be seen below.

4.3 Dependence of chlorfenvinphos mobility on its volume fraction in the cuticle and the mechanism of adjuvant action on solute mobility

The above argument makes it likely that data in Fig. 3B do not reflect the real effectiveness of chlorfenvinphos as an accelerator. Average volume fractions are plotted, but the volume fractions effective will be those in the wax of the limiting barrier, which are likely to be much smaller. The lines of the desorption graphs at different volume fractions go through the origin only with the lowest volume fraction. After starting desorption, chlorfenvinphos volume fractions in the cuticles decrease and rates should be maximal at the beginning. This decrease was observed only for the higher volume fractions. Since desorption was started one day after loading the CM with chlorfenvinphos, it appears that, at the higher volume fraction leading to much more rapid diffusion, a

fraction of the applied chlorfenvinphos probably had penetrated to the outer surface of the CM (see above). This superficial fraction is immediately desorbed when the PLS is added and this could account for the positive y-intercepts. For these reasons, we decided to use volume fractions estimated for the time 65 h (see Fig. 3A) for further data analysis.

The free-volume theory of Fujita[39] suggests that diffusion coefficients in polymers depend on the fractional free volume of the polymer which can be occupied by the diffusant molecules. At low volume fractions, diffusion coefficients of penetrants depend exponentially on the volume fraction X_v [16] occupied by the penetrant. Since diffusion coefficients are proportional to rate constants k^*,[22] we can write this as

$$k^* = k_0^* \exp(\alpha X_v) \quad (3)$$

where k_0^* is the rate constant at zero volume fraction obtained by extrapolation to zero and α is an empirical constant inversely proportional to temperature. The parameter α reflects interactions between penetrant and polymer and is a measure of the free volume of the polymer.[39] However, eqn (3) is valid only for small volume fractions of the order of 0·01. Our average volume fractions of CFP are much higher and this may explain the non-linearity of the graphs in Fig. 3B, which should give a straight line when eqn (3) is obeyed. Due to non-linearity we cannot accurately estimate k_0^*.

For a wider range of volume fractions, the dependence of diffusion coefficients on volume fraction in polymers is given by a relation which is again written using rate constants instead of diffusion coefficients[16,39]

$$k^* = k_0^* \exp(AX_v/(B + X_v)). \quad (4)$$

A and B are empirical constants and k_0^* has the same meaning as in eqn (3). The constant A is proportional to the free volume of the pure polymer, while B is proportional to the free volume of the pure polymer and to the increase of free volume by the action of the dispersed diffusant.[39] Rearranging eqn (4) and taking the logarithm gives

$$1/\log(k^*/k_0^*) = 1/A' + B/A' \cdot 1/X_v. \quad (5)$$

with $A' = A/2{\cdot}3$. Before we can proceed we need an estimate of k_0^*. If it is assumed that eqn (3) is valid for the three lowest volume fractions, the rate constant at zero volume fraction k_0^* can be calculated as indicated by the dashed line in Fig. 3B. This value is an overestimate but the exact value of k_0^* is not important in the present context. We simply need a reference value for applying eqn (5) to obtain information about the relative change of k^* with a change in the average volume fraction of chlorfenvinphos.

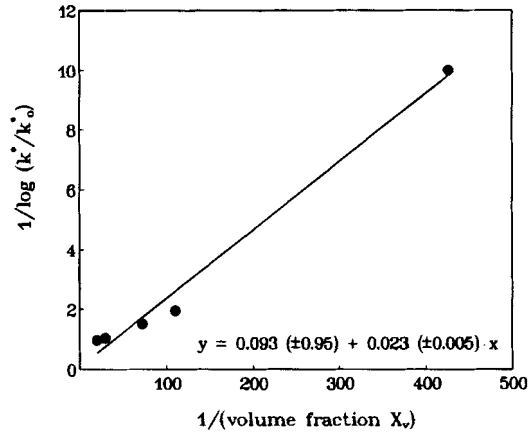

Fig. 6. Double reciprocal plot relating the increase in chlorfenvinphos mobility to its volume fraction sorbed in pear leaf CM (see text).

Plotting the data according to eqn (5) resulted in a straight line (Fig. 6), showing that this equation is applicable for describing the concentration dependence of chlorfenvinphos diffusion across pear leaf cuticle. The great distance of the single data point on the upper right corner (representing the lowest volume fraction) and the cluster on the lower left corner results from plotting inverse volume fractions. The volume fractions themselves are evenly distributed (0·002, 0·009, 0·014, 0·034, 0·051; *cf.* Fig. 3A). When fitting the line, we included the lowest volume fraction because the low volume fractions are of practical significance and cuticles are not overloaded with chlorfenvinphos, as might be the case with the two highest volume fractions. The exponential term of eqn (4) describes a saturation behaviour of chlorfenvinphos sorption of a Langmuir type for the change of k^* with volume fraction. This indicates that chlorfenvinphos is homogeneously dispersed in the region limiting velocity of diffusion, which is the limiting skin of pear leaf CM. However, we do not know if the limiting skin consists of a thin wax layer on top of the cuticle or if it is a wax/cutin composite. Possible sides of sorption in both cases are the interfaces between crystalline waxes and noncrystalline regions. In any event conformation of the data to eqn (5) indicates a homogeneous distribution in the limiting skin, not a formation of a continuous phase made up of neat chlorfenvinphos. These conclusions are consistent with our earlier suggestion that temperature affects the viscosity of amorphous waxes and cutin but not cuticle structure.[40]

Effects of accelerators and temperatures have a common base, they both increase solute mobility by decreasing the viscosity of the limiting barrier. This has far-reaching practical consequences. Rates of foliar penetration decrease rapidly with decreasing temperatures, because activation energies are very high.[40] Temperature coefficients, Q_{10}, amount to more than 4 and are higher than commonly found for metabolic pro-

cesses. Thus, systemic pesticides and growth regulators penetrate very slowly and this could account for the observation that, for instance, chemical thinning is difficult if cold weather prevails during and after spraying.[41,42] This difficulty could be overcome by using special low-temperature formulations containing accelerator adjuvants that restore rates of penetration to values observed at higher temperatures. Alternatively, suitable accelerator adjuvants could be added to the tank mixture.

Riederer *et al.*[30] have analysed published data on the effects of fatty alcohols and alcohol ethoxylates differing in the chain lengths of the alkyl and ethoxy moieties on the diffusion of 2,4-D across *Citrus* leaf cuticles. They found that acceleration effects due to these adjuvants were correlated with their volume fractions in the cuticle. We have tested if eqn (5) can be applied to these data. The effects of adjuvants on diffusion of 2,4-D are the ratios of the rate-constants of desorption in the presence and absence of adjuvants.[29] These effects were used to calculate the left hand term of eqn (5), which was plotted against the average volume fractions of adjuvants in the cuticles (Fig. 7). From the 26 compounds two ($C_{14}E_7$ and $C_{16}E_8$, with C being the number of carbon atoms in the alcohol and E being the number of ethoxy groups) were omitted because maximum effects were not reached with these.[30] The graph obtained is reasonably linear, showing that the theory underlying eqn (5) can also account for the effects on 2,4-D mobility of fatty alcohols and ethoxylated fatty acids. The slopes of the lines in Figs 6 and 7 give the relative change of diffusant mobility with volume fraction and can be used to compare the efficiencies of different classes of adjuvants included in pesticide formulation if the parameter is estimated using the same plant species and active ingredient. The slopes (B/A' in eqn (5)) are significantly different having values of 0·023 (±0·005, 95% CI) for chlorfenvinphos in pear leaf CM compared to 0·039 (±0·006) for 2,4-D in *Citrus* and fatty alcohol ethoxylates. Since CM from different plant species were used it is not possible to decide to which extent wax and adjuvant properties contributed to these differences in slopes.

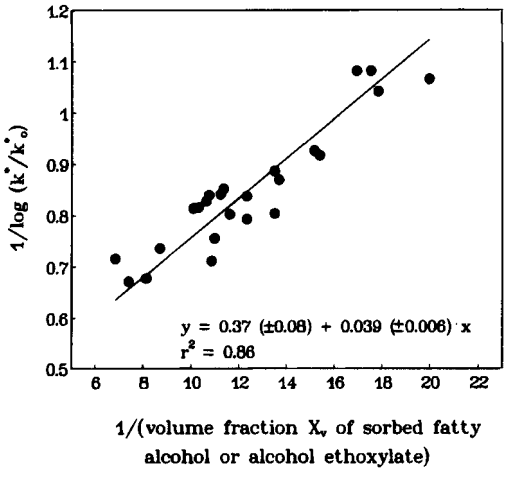

Fig. 7. Double reciprocal plot relating the increase in 2,4-D mobility to the volume fraction of sorbed fatty alcohol or alcohol ethoxylates in *Citrus* leaf CM (see text).

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